

Growth, Antioxidant Enzymes Activities and Photosynthetic Pigments of Two Rice (*Oryza sativa* L.) Cultivars as Influenced by Application of Gibberellic Acid and Sodium Arsenate

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ABSTRACT

The effects of gibberellic acid (GA₃) and sodium arsenate on enzymatic antioxidants activities (superoxide dismutase, guaiacol peroxidase and catalase), soluble proteins content, growth and photosynthetic pigments (chl_a, chl_b, total chl. and carotenoids) of 'Tarom' and 'Shiroudi' rice were investigated. Thirty-days-old seedlings were treated with different concentrations of sodium arsenate (0, 50 and 100 μM) and GA₃ (0 and 10 μM) for two weeks. The results showed that sodium arsenate increased the activities of the enzymatic antioxidants and protein content significantly and reduced the growth parameters, chl_a, chl_b, and carotenoids contents in both cultivars of rice. Meanwhile, GA₃ increased the activities of the enzymatic antioxidants, protein content, growth parameters, chl_a, chl_b, and carotenoids contents significantly in both cultivars of rice treated with sodium arsenate. In general, the results of this study showed that GA₃ increased the resistance of the two examined rice cultivars treated with sodium arsenate.

Keywords: Rice, enzymatic antioxidants, gibberellic acid, photosynthetic pigments, sodium arsenate, soluble proteins.

Abbreviations: GA₃ (gibberellic acid), CAT (catalase), POX (peroxidase), SOD (superoxide dismutase)..

INTRODUCTION

Arsenic is spread out broadly by soil materials, air and human activities in environment (Singh *et al.*, 2009). This heavy metal is presented in rock, soil, water and air in organic and inorganic forms (Rintala *et al.*, 2014). High content of arsenic in some farm soils is due to industrial and agricultural activities decline soil fertility (Miteva *et al.*, 2005). Water pollution containing arsenic has been worrying in many countries including Argentina, Australia, Bangladesh, Chile, China, Hungary, India, Mexico, Peru, Thailand and USA (Chan *et al.*, 2013).

However, in anaerobic soils, arsenate (AsV) is the constant form and in reducing condition, arsenite (AsIII) is the dominant species that both are highly toxic for plants. Arsenate is a phosphate analog and it can compete with phosphate in the cytoplasm as a replacement of phosphate in ATP declining energy flows in cells. Arsenic is extremely toxic in plants because, it reacts with sulfhydryl groups in enzymes, their cofactors and proteins. High arsenic concentrations change the activity of several enzymes dealing in metabolism of plants (Singh *et al.*, 2009).

Arsenic transport is depended on pH, arsenic species, redox conditions, inorganic and organic forms of arsenic (Polizzotto *et al.*, 2013). Moreover, plants have several mechanisms against arsenic stress including high affinity phosphate/ arsenate transport system, compartmentalization, reduction and translocation of

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arsenic. Also, phytohormones can induce resistance mechanisms that may provide a strategy to raise the tolerance. GA inhibits the effects of abiotic stress and decreases the accumulation of heavy metals in plant (such as broad bean), and increases antioxidant activities scavenging reactive oxygen species (ROS) produced in plants under stress conditions (Zhu *et al.*, 2012).

It has been found that gibberellin has a positive effect on plant growth and development (Tuna *et al.*, 2008; Bartoliet *al.*, 2013). Regarding the effect of sodium arsenate on the growth and activity of oxidizing enzymes, Swarnakar (2014) reported that pretreatment with GA₃ increased the resistance of mungbean seedlings against As stress. Also, Ghosh *et al.* (2015) found that gibberellic acid was able to dominate the toxic effects of sodium chloride stress in mungbean (*Vignaradiata* L. Wilczek). The current study aimed to investigate the impact of gibberellic acid on the resistance of two rice cultivars, 'Tarom' and 'Shiroudi' under sodium arsenate stress.

MATERIAL AND METHODS

Plant materials and treatments

Seeds of 'Tarom' and 'Shiroudi' rice were prepared from the Center of Rice Research in Amolcity, Mazandaran, Iran. Experiments were conducted in Paddy fields in the city of Sari at the north of Iran, at latitude of 36.33 and longitude 53 and at height of 25.70 meters of sea level in 2013 in 4 replicates. The seeds were sterilized in 10% (v/v) sodium hypochlorite for 10 min and then transferred in wet soil condition for germination. The 25 days-old seedlings with 2-3 leaves were transplanted in pots (4 in each pot) filled with field soil. Soil chemical and physical properties of the filled were analyzed (Table 1). The 30 days-old seedlings were treated with three concentrations of sodium arsenate (0, 50 and 100 μ M) and two concentrations of gibberellic acid (0 and 10 μ M). After two weeks of treatment, the seedlings were harvested for analyzing.

Preparation of plant extracts

For the examination of soluble protein and enzymatic antioxidants activities, 0.1 g of fresh leaf and root tissue were grinded separately in liquid nitrogen and 0.1 M buffer phosphate of potassium (pH 6.8). The extracts were centrifuged separately at 12,000 rpm for 20 min at 4°C.

Soluble protein assay

After centrifugation, the above extract was carefully transferred to another microtube. Leaf and root soluble protein content of 'Tarom' and 'Shiroudi' rice were examined using spectrophotometer (UV/Vis) at wavelength of 595 nm, according to the method of Bradford (1976) with bovine serum albumin as a standard.

Enzymatic antioxidants activities assay

Peroxidase (POD) activity was measured using Dazy *et al.* (2008) method, Catalase (CAT) activity using Johansson and Borg (1988) method, and Superoxide Dismutase (SOD) activity of leaves and roots of the two rice cultivars (Tarom and Shiroudi) were examined using the method of Giannopolitis and Ries (1977).

Growth parameters assay

To measure the fresh weight of the plant shoots and roots, the roots and shoots were separated and then weighted using an accurate balance. Also, root length, stem length and leaf length and leaf width were measured (Evans and Hughes, 1962). Dry weight of the plant shoots and roots were measured after 24 h drying in oven at 70°C.

Photosynthetic pigments assay

The amounts of photosynthetic pigments were determined using Lichtenthaler (1987) method. Pigments were extracted from leaves with 80% acetone, the extracts filtered with Whatman filter paper, and then the pigments were examined using spectrophotometer (UV/Vis) at wavelengths of 470, 646.8 and 663.2 nm. The

amounts of photosynthetic pigments were quantified using the following formula:

$$\text{Chl. } a = (12.25 A_{663.2} - 2.79 A_{646.8})$$

$$\text{Chl. } b = (21.21 A_{646.8} - 5.1 A_{663.2})$$

$$\text{Chl. T} = \text{Chl. } a + \text{Chl. } b$$

$$\text{Car.} = (1000 A_{470} - 1.8 \text{Chl. } a - 85.02 \text{Chl. } b/198)$$

Statistical analysis

A completely randomized design with four replicates was used in each experiment. The means were separated by Duncan's multiple range test (DMRT) using SPSS (version 16.0) and presented as mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

Soluble protein content

The effect of sodium arsenate (Na_2HAsO_4) and gibberellic acid (GA_3) on soluble protein content of leaves and roots in 'Tarom' and 'Shiroudi' rice compared to control is presented in tables 2 and 3. According to the results, soluble protein content in leaves and roots increased significantly at both sodium arsenate concentrations for the two cultivars. However, the increase in 'Shiroudi' was clearer than 'Tarom' under sodium arsenate treatment. Moreover, soluble protein content of leaves and roots increased significantly at treatments of GA_3 and sodium arsenate compared with control in both examined rice cultivars. Environmental stresses such as heavy metals have negative impacts on protein content of plants. The results of the current study showed that sodium arsenate increased soluble protein content of 'Tarom' and 'Shiroudi' rice (Tables 2 and 3). Similar results have been found in *Zeamays* L. treated with lead (Pb) (Hussain *et al.*, 2013) and in *Nasturtium officinale* exposed to arsenite (Ozturket *et al.*, 2010).

Soluble protein content is an important indicator of physiological status of plants. This increase in protein

content induces adaptive responses of plants such as increasing antioxidative activities and proline content. The increase in protein content can be attributed to increasing enzymatic proteins synthesis e.g. antioxidants to confront the negative impacts under stress conditions (Mittal *et al.*, 2012).

According to the results of this study, sodium arsenate along with GA_3 treatments significantly increased soluble protein content in both examined rice cultivars. Similar results were reported in sugarcane plants exposed to gibberellic acid under Pb, Cu and Cd stress in green algae of *Chlorella vulgaris* (Piotrowska-Niczyporuk *et al.*, 2012) and in *Triticum aestivum* L. under Zn, Pb and Cd stress (Ergün and Öncel, 2012).

Peroxidase activities

Sodium arsenate treatment increased peroxidase (POX) activity of leaves and roots of both rice cultivars, and this impact was significant at 100 μM sodium arsenate treatment. Meanwhile, using GA_3 along with sodium arsenate significantly increased POX activity of leaves and roots in both examined rice cultivars, and the increase in 'Tarom' leaves of was more than that of 'Shiroudi' (Tables 2 and 3). The POX activity in the roots and leaves of 'Tarom' and 'Shiroudi' rice (Tables 2 and 3) showed that sodium arsenate and gibberellic acid significantly increased POX activity. Increased POX activity under heavy metals stress had been reported by Chaouiet *et al.* (1997) in bean (*Phaseolus vulgaris* L.) under Cd and Zinc stress and Choudhary *et al.* (2012) in *Raphanus sativus* L. under Cr stress.

Increasing POX activity was used as a biomarker of stress that might be due to localization of peroxidase in cell walls because, it contribute in lignin biosynthesis as a physical inhibition against toxic materials (Erdalet *et al.*, 2011). Moreover, GA_3 increased POX activity in roots and leaves of both rice cultivars and this impact in the leaves was more than roots. These results are in

agreement with the results found in in cherry tomato (*Solanumlycopersicum* L.) fruit treated with GA under chilling injury stress (Ding *et al.*, 2015) and in maize treated with GA₃ under salinity stress (Tuna *et al.*, 2008).

Catalase activity

The effect of sodium arsenate and GA₃ on leaf and root catalase activity in 'Taron' and 'Shiroudi' rice compared to control is presented in tables 2 and 3. Sodium arsenate significantly increased CAT activity of leaves and roots of both rice cultivars at 50 and 100 μM concentrations; however, this impact at 100 μM was greater than 50 μM. Also, the treatment of sodium arsenate along with GA₃ significantly increased CAT activity of leaves and roots in both rice cultivars. In this study, the increased in CAT activity at both concentrations of sodium arsenate indicating the role of CAT in detoxification of H₂O₂ under arsenic stress (Tables 2 and 3). This finding is in an agreement with the results of Wang *et al.* (2012) in *Vallisnerianatans* under Pb stress, and Zhang *et al.* (2007) in *Kandeliacandel* and *Bruguieragymnorrhiza* seedlings treated with Pb²⁺, Cd²⁺, and Hg²⁺.

Catalase is the main antioxidant enzymes that eliminate H₂O₂ in the mitochondria and microbodies (Liu *et al.*, 2009). It is an important scavenger of H₂O₂ produced during photorespiration and stress conditions (Dazyet *et al.*, 2009) and convert H₂O₂ into H₂O and O₂ through two electrons transfer and prevent the generation of OH[•] and consequently protect proteins, nucleic acids and lipids against ROS (Gallego *et al.*, 1996). The current results showed that GA₃ increased CAT activity and improved the resistance of the plants against active oxygen radicals produced under sodium arsenate stress. Similar results had been reported in *Dracocephalum moldavica* L. (Rezaei *et al.*, 2013), *Brassica juncea* (Yusuf *et al.*, 2008) and in green algae of *Chlorella vulgaris* treated with gibberellin under Pb,

Cd and Cu stress (Piotrowska-Niczyporuk *et al.*, 2012).

Superoxide dismutase activity

According to tables 2 and 3, the activities of leaf superoxide dismutase (SOD) of both cultivars increased significantly under sodium arsenate and GA₃ treatments, but the impact was not significant in roots of both rice cultivars. The SOD activity of leaves and roots of both cultivars increased significantly at 100 μM sodium arsenate concentration along with GA₃ treatment. The results of this study indicated that SOD activity increased with increasing sodium arsenate concentration in roots and leaves of both rice cultivars (Tables 2 and 3). Also, according to the reports, heavy metals (Cd, Co, Pb, Cu, As and Ag) increased SOD activity in *Aeluropus littoralis* (Rastgoo and Alemzadeh, 2011) as well as in *Lupinus luteus* L. (Jomová and Morovič, 2009). Destructive physiological and anatomical impacts would occur in plants that the capacity of antioxidant processes and detoxification mechanisms are lower than the amount of ROS production (Szöllösi, 2014).

Superoxide dismutase is the first enzyme in ROS detoxifying process that converts O₂⁻ to H₂O₂ in cytosol, chloroplast and mitochondria and plays as a defense mechanism against OH[•] formation (Liu *et al.*, 2009). Increased SOD activity in both concentrations of sodium arsenate in rice cultivars indicates the high production of ROS under heavy metals stress (Levent Tuna *et al.*, 2008). Increased activity of SOD enzyme under sodium arsenate stress treated with GA improved plants resistance against oxidative stress. Similarly, It was found that gibberellin increased the activity of SOD in *Triticum aestivum* L. under Ni treatment (Siddiqui *et al.*, 2011) and in cherry tomato (*Solanumlycopersicum* L.) fruit under chilling stress (Ding *et al.*, 2015).

Growth parameters

Based on the results of this study, the fresh and dry weight of roots and leaves, stem length, root length and

leaf width decreased significantly at both sodium arsenate concentrations in both cultivars of rice (Tables 4, 5); and this negative impact at 100 μM was greater than 50 μM . However, the growth parameters significantly increased at 100 μM concentration of sodium arsenate compared with 50 μM when the seedlings treated with gibberellin (Tables 4, 5). High temperatures, salinity, drought and heavy metals have negative effect on fresh weight and dry weight of shoots and roots as well as the metabolism and physiological activities of plants (Mathieu *et al.*, 2014; Hayat Bhatti *et al.*, 2013; Nadeem *et al.*, 2014).

Based on the results of this study, sodium arsenate reduced fresh weight and dry weight of roots and leaves and other growth parameters in of both examined cultivars of rice (Tables 4 and 5). Similar results were observed in *Asteriscusmaritimus* treated with NaCl under water stress (Sánchez-Rodríguez *et al.*, 2012). The balance between generation and degradation of ROS were required to protect metabolic functions under these stress conditions to avoid the oxidative injuries. Plants utilize most of their resources under stress conditions to improve their defense mechanisms for growth and development (Howladar, 2014). Also, heavy metals can reduce cell division and growth of stems, roots and in this situation, gibberellin can have a positive impact on plant growth and development (Levent Tuna *et al.*, 2008; Bartoliet *et al.*, 2013). The current results were in agreement with those of Shaddadet *et al.* (2011) in maize treated with gibberellin under drought stress, Khanet *et al.* (2011) in *Aspergillusfumigatus* sp. treated with gibberellin under salinity stress and Ratushnyaket *et al.* (2012) in *Pisumsativum* treated with salicylic acid under Pd stress.

Photosynthetic pigments

According to table 3, in both cultivars of rice, the content of chlorophylls a and b, total chlorophyll and

carotenoids of leaves decreased in both sodium arsenate concentrations, and this impact at 100 μM was greater than 50 μM ; however, this decline was not significant for chlorophyll b. Sodium arsenate along with GA_3 treatment increased significantly the amount of chlorophyll a and b, total chlorophyll and carotenoids in both examined rice cultivars. Reduction of the number of chloroplasts, destruction of photosynthetic pigment and reduction of pigment synthesis would occur under stress conditions. Heavy metal stress destroys the structure of chloroplast, grana, thylakoid and also imbalance the pigment protein complex (Miteva *et al.*, 2005). Arsenic has negative impact on photosynthetic pigments of the plants (Azizur-Rahman *et al.*, 2007). In this study, sodium arsenate reduced leaf photosynthetic pigment in both cultivars of rice (Table 6). Similar effect had been reported in *Glauciumflavum* Crantz treated with Cu (Cambrollé *et al.*, 2011), in rice (*Oryza sativa* L.) treated with arsenic (Azizur Rahman *et al.*, 2007), and in barley treated with Cd (Vassilev *et al.*, 2004).

Siddiqui *et al.* (2011) found that gibberellin increased the number of chloroplasts in wheat plants under Ni stress. Phytohormones increase antioxidant activity under heavy metal stress and reduce ROS levels and increase the resistance against heavy metals (Piotrowska-Niczyporuket *et al.*, 2012). The current results regarding the positive impacts of gibberellin on photosynthetic pigments in both cultivars of rice under sodium arsenate treatment are in agreement with the results of Piotrowska-Niczyporuket *et al.* (2012) in *Chlorella vulgaris* treated with gibberellin under Pb and Cu stress and Anuradha and Seeta-Ram-Rao (2003) in rice seeds (*Oryza sativa* L.) treated with brassinosteroids and salt stress.

CONCLUSION

According to the results, sodium arsenate treatment increased the antioxidant activities (CAT, POX and

SOD) and soluble protein content. Growth parameters and photosynthetic pigments (chl a and b , total chlorophyll and carotenoids) reduced in 'Tarom' and 'Shiroudi' rice. GA $_3$ treatment increased soluble protein content, antioxidant activities, growth parameters and photosynthetic pigments and enhanced the resistance of rice under sodium arsenate stress. Furthermore, this

study showed that GA $_3$ increased the resistance of the two examined rice cultivars treated with sodium arsenate.

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Table 1. Chemical and physical properties of the soil used in the experiment.
CCE (Calcium Carbonate Equivalent), EC (Electric conductance).

Depth (Cm)	0-30	CCE (%)	30
Texture	Clay	Organic carbon (%)	2
Clay (%)	36	Organic matter (%)	3.44
Silt (%)	28	Total nitrogen (%)	0.15
Sand (%)	36	Phosphorus (p.p.m)	26.7
EC	1.43	Potassium (p.p.m)	294
pH	7.26	Arsenic (p.p.m)	<10

Table 2. Effect of gibberellic acid and sodium arsenate on soluble protein contents, peroxidase, catalase and superoxide dismutase activities in leaves of two rice cultivars.

Cultivar	Sodium arsenate (μ M)	GA $_3$ (μ M)	Soluble proteins (mg g $^{-1}$ F.W.)	POX (Δ ODg $^{-1}$ F.W min $^{-1}$)	CAT (Δ ODg $^{-1}$ F.W min $^{-1}$)	SOD (U mg $^{-1}$ protein)
Tarom	0	0	505.4 \pm 33 ^f	21.91 \pm 0.51 ^{fg}	34.56 \pm 3.26 ^{fg}	228.7 \pm 10.0 ^e
		10	1328 \pm 174 ^e	26.75 \pm 1.77 ^{ef}	50.78 \pm 1.29 ^{de}	337.8 \pm 15.3 ^d
	50	0	780.9 \pm 81.7 ^f	29.83 \pm 2.16 ^{de}	62.1 \pm 2.76 ^{cd}	791.3 \pm 8.856 ^a
		10	1504 \pm 165 ^{cde}	37.917 \pm 1.91 ^{bc}	66.48 \pm 3.80 ^c	772.8 \pm 20.5 ^a
	100	0	1452 \pm 90.8 ^{cde}	41 \pm 2.54 ^{ab}	80.24 \pm 6.32 ^{ab}	584.6 \pm 61.5 ^b
		10	1877 \pm 106 ^{abc}	45.52 \pm 2.19 ^a	91.08 \pm 1.86 ^a	777.5 \pm 29.2 ^a
Shiroudi	0	0	1424 \pm 131 ^{de}	3.3 \pm 0.17 ^d	26.25 \pm 2.66 ^g	497.7 \pm 9.65 ^{bc}
		10	1565 \pm 175 ^{bcde}	4.05 \pm 0.15 ^d	41.08 \pm 5.46 ^{ef}	522.7 \pm 32.8 ^{bc}
	50	0	2260 \pm 178 ^a	4.35 \pm 0.15 ^d	36.12 \pm 2.53 ^{fg}	487.3 \pm 17.6 ^c
		10	1818 \pm 146 ^{bcd}	6.15 \pm 0.56 ^c	43.77 \pm 4.55 ^{ef}	728.3 \pm 40.6 ^a
	100	0	1498 \pm 144 ^{cde}	6.9 \pm 0.79 ^{bc}	47.00 \pm 4.03 ^{ef}	762.5 \pm 34.8 ^a
		10	1950 \pm 86.9 ^{ab}	7 \pm 0.14 ^{bc}	69.23 \pm 7.69 ^{bc}	735.8 \pm 13.7 ^a

Table 3. Effect of gibberellic acid and sodium arsenate on protein contents, peroxidase, catalase and superoxide dismutase activities in roots of two rice cultivars.

Cultivar	Sodium arsenate (μM)	GA ₃ (μM)	Protein (mg g ⁻¹ F.W.)	POX (ΔOD^{-1} F.W min ⁻¹)	CAT (ΔODg^{-1} F.W min ⁻¹)	SOD (U mg ⁻¹ protein)
Tarom	0	0	308.6 \pm 46.7 ^{ef}	3.3 \pm 0.3 ^d	28.68 \pm 1.36 ^{ef}	1527 \pm 131 ^d
		10	332.5 \pm 24.8 ^{ef}	4.2 \pm 0.24 ^d	35.32 \pm 2.31 ^e	3765 \pm 226 ^c
	50	0	388.6 \pm 36.8 ^e	4.05 \pm 0.37 ^d	47.71 \pm 4.61 ^{cd}	1876 \pm 67.4 ^d
		10	549.7 \pm 45.7 ^d	7.65 \pm 0.61 ^b	62.4 \pm 1.28 ^{ab}	2067 \pm 67.4 ^d
	100	0	214.7 \pm 23.9 ^{fg}	6.9 \pm 0.38 ^{bc}	54.97 \pm 2.97 ^{bc}	3547 \pm 170 ^c
		10	546.1 \pm 45.7 ^d	10.4 \pm 0.37 ^a	49.04 \pm 2.97 ^{cd}	2067 \pm 162 ^d
Shiroudi	0	0	90.47 \pm 10.3 ^g	3.3 \pm 0.17 ^d	24.55 \pm 2.592 ^f	6280 \pm 353 ^b
		10	724 \pm 54.7 ^c	4.05 \pm 0.15 ^d	45.325 \pm 4.038 ^d	7592 \pm 536 ^a
	50	0	601.2 \pm 60.6 ^{cd}	4.35 \pm 0.15 ^d	31.567 \pm 2.375 ^{ef}	7605 \pm 593 ^a
		10	1177 \pm 42.7 ^b	6.15 \pm 0.56 ^c	58.3 \pm 2.875 ^{ab}	5893 \pm 285 ^b
	100	0	221.1 \pm 53 ^{fg}	6.9 \pm 0.79 ^{bc}	43.625 \pm 3.064 ^d	6398 \pm 449 ^b
		10	1529 \pm 48.9 ^a	7 \pm 0.14 ^{bc}	64.3 \pm 2.002 ^a	6341 \pm 449 ^b

Table 4. Effect of gibberellic acid and sodium arsenate on fresh and dry weight of shoots and roots of two rice cultivars.

Cultivar	Sodium arsenate (μM)	GA ₃ (μM)	Fresh weight shoots(g/plant)	Fresh weight (g/plant)roots	Dry weight shoots (g/plant)	Dry weight roots (g/plant)
Tarom	0	0	4.64 \pm 0.24 ^a	1.569 \pm 0.19 ^a	0.746 \pm 0.05 ^{bc}	0.178 \pm 0.01 ^b
		10	4.807 \pm 0.10 ^a	1.607 \pm 0.13 ^a	0.886 \pm 0.05 ^a	0.226 \pm 0.01 ^a
	50	0	4.362 \pm 0.22 ^a	1.203 \pm 0.07 ^b	0.581 \pm 0.02 ^d	0.146 \pm 0.008 ^c
		10	4.562 \pm 0.15 ^a	1.391 \pm 0.05 ^{ab}	0.805 \pm 0.05 ^{ab}	0.197 \pm 0.007 ^{ab}
	100	0	1.91 \pm 0.20 ^e	0.489 \pm 0.06 ^d	0.450 \pm 0.04 ^e	0.056 \pm 0.007 ^{gh}
		10	3.85 \pm 0.16 ^b	1.24 \pm 0.03 ^b	0.670 \pm 0.03 ^{cd}	0.131 \pm 0.019 ^{cd}
Shiroudi	0	0	2.435 \pm 0.26 ^d	0.685 \pm 0.07 ^{cd}	0.396 \pm 0.02 ^{ef}	0.083 \pm 0.009 ^{efg}
		10	2.918 \pm 0.20 ^c	0.860 \pm 0.06 ^c	0.615 \pm 0.02 ^d	0.107 \pm 0.004 ^{de}
	50	0	1.262 \pm 0.03 ^{fg}	0.609 \pm 0.04 ^c	0.306 \pm 0.02 ^{fg}	0.061 \pm 0.006 ^{fgh}
		10	1.6 \pm 0.17 ^{fg}	0.741 \pm 0.05 ^{cd}	0.441 \pm 0.03 ^e	0.096 \pm 0.008 ^e
	100	0	0.828 \pm 0.06 ^g	0.241 \pm 0.02 ^e	0.249 \pm 0.04 ^g	0.037 \pm 0.005 ^h
		10	1.651 \pm 0.05 ^{ef}	0.546 \pm 0.03 ^d	0.331 \pm 0.02 ^{efg}	0.092 \pm 0.004 ^{ef}

Table 5. Effect of gibberellic acid and sodium arsenate on stem length, leaf length, leaf width and root length of two rice cultivars.

Cultivar	Sodium arsenate (μM)	GA ₃ (μM)	Stem length(cm)	Leaf length (cm)	Leaf width(mm)	Root length (cm)
Tarom	0	0	29±2.79 ^{ab}	41±20.04 ^b	10.5±0.95 ^a	13.375±1.51 ^f
		10	32±1.22 ^a	54.75±2.75 ^a	10.75±0.47 ^a	25.75±1.65 ^{ab}
	50	0	24.5±1.70 ^c	40±1.87 ^{bc}	7.75±0.47 ^{cd}	20.75±1.03 ^{cd}
		10	31.5±0.64 ^a	58±2.34 ^{ab}	9.75±0.47 ^{ab}	21±1.87 ^{cd}
	100	0	23.75±1.18 ^{cd}	34.75±1.10 ^{cd}	6±0.40 ^{def}	15.25±1.10 ^{ef}
		10	30±0.70 ^a	45±2.73 ^b	8.75±0.47 ^{bc}	29.5±0.64 ^a
Shiroudi	0	0	22.75±0.85 ^{cd}	40.75±1.49 ^b	6.75±0.47 ^{de}	24.75±1.43 ^{bc}
		10	25.75±0.85 ^{bc}	44.5±1.19 ^b	7.5±0.64 ^{cde}	27.5±1.19 ^{ab}
	50	0	20.25±0.98 ^{de}	34.5±2.32 ^{bc}	5.75±0.47 ^{ef}	18.5±1.19 ^{de}
		10	22.75±0.74 ^{cd}	41.25±1.31 ^b	7.75±0.47 ^{cd}	26±0.81 ^{ab}
	100	0	18.25±0.47 ^e	30±1.08 ^d	5±0.57 ^f	17±1.82 ^{def}
		10	23.5±0.64 ^{cd}	40.25±0.47 ^{bc}	7.25±0.47 ^{cde}	25.25±1.18 ^{ab}

Table 6. Effect of gibberellic acid and sodium arsenate on photosynthetic pigments of two rice cultivars.

Cultivar	Sodium arsenate (μM)	GA ₃ (μM)	Chlorophyll <i>a</i> (mg g ⁻¹ F.W)	Chlorophyll <i>b</i> (mg g ⁻¹ F.W)	Totalchl. (mg g ⁻¹ F.W)	Carotenoids (mg g ⁻¹ F.W)
Tarom	0	0	1.808±0.10 ^{cd}	0.793±0.04 ^{cde}	2.601±0.14 ^{cd}	0.438±0.01 ^{cde}
		10	2.709±0.18 ^a	1.263±0.08 ^a	3.972±0.27 ^a	0.575±0.05 ^a
	50	0	1.632±0.06 ^{cd}	0.722±0.02 ^e	2.355±0.08 ^{cde}	0.382±0.01 ^{ef}
		10	2.227±0.07 ^b	0.973±0.03 ^{cd}	3.201±0.11 ^b	0.544±0.01 ^{ab}
	100	0	1.479±0.007 ^{de}	0.668±0.005 ^e	2.148±0.004 ^{de}	0.353±0.006 ^f
		10	1.878±0.01 ^c	0.769±0.009 ^{de}	2.648±0.01 ^{cd}	0.460±0.01 ^{bcde}
Shiroudi	0	0	1.869±0.12 ^c	0.864±0.07 ^{cde}	2.736±0.20 ^{bc}	0.472±0.02 ^{bcd}
		10	2.594±0.07 ^a	1.175±0.06 ^{ab}	3.776±0.14 ^a	0.538±0.001 ^{ab}
	50	0	1.827±0.21 ^c	0.81±0.11 ^{cde}	2.639±0.32 ^{cd}	0.411±0.05 ^{def}
		10	2.218±0.06 ^b	1.00±0.019 ^{bc}	3.226±0.08 ^b	0.523±0.001 ^{abc}
	100	0	1.189±0.05 ^e	0.689±0.006 ^e	1.875±0.06 ^e	0.111±0.005 ^g
		10	1.859±0.13 ^c	0.747±0.12 ^e	2.674±0.20 ^{cd}	0.421±0.03 ^{def}

REFERENCES

- Anuradha, S., Seeta-Ram-Rao, S., 2003. Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity. *Plant Growth Regulation*. 40(1): 29–32.
- Azizur-Rahman, M., Hasegawa, H., Mahfuzur-Rahman, M., Nazrul Islam, M., Majid-Miah, M.A., Tasmien, A., 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere*. 67: 1072–1079.
- Bartoli, C.G., Casalongué, C.A., Simontacchi, M., Marquez-Garcia, B., Foyer, C.F., 2013. Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. *Environmental and Experimental Botany*. 94: 73–88.
- Bradford, M., 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248–254.
- Cambrollé, J., Mateos-Naranjo, E., Redondo-Gómez, S., Luque, T., Figueroa, M.E., 2011. Growth, reproductive and photosynthetic responses to copper in the yellow-horned poppy, *Glaucium flavum* Crantz. *Environmental and Experimental Botany*. 71: 57–64.
- Chaoui, A., Mazhoudi, S., Ghorbal, M.H., El Ferjani, E., 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Science*. 127(2): 139–147.
- Chan, W.F., Li, H., Wu, F.Y., Wu, S.C., Wong, M.H., 2013. Arsenic uptake in upland rice inoculated with a combination or single arbuscular mycorrhizal fungi. *Journal of Hazardous Materials*. 262: 1116–1122.
- Choudhary, S.P., Kanwar, M., Bhardwaj, R., Yu, J.Q., Tran, L.S.P., 2012. Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in *Raphanus sativus* L. *Public Library of Science*. 7(3): 332–342.
- Dazy, M., Masfaraud, J.F., Féraud, J.F., 2009. Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. *Chemosphere*. 75(3): 297–302.
- Dazy, M., Jung, V., Ferard, J.F., Masfaraud, J.F., 2008. Ecological recovery of vegetation on a coke-factory soil: role of plant antioxidant enzymes and possible implication in site restoration. *Chemosphere*. 74: 57–63.
- Ding, Y., Sheng, J., Li, S., Nie, Y., Zhao, J., Zhu, Z., 2015. The role of gibberellins in the mitigation of chilling injury in cherry tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*. 10: 88–95.
- Erdal, S., Aydın, M., Genisel, M., Taspınar, M.S., Dumlupınar, R., Kaya, O., Gorcek, Z., 2011. Effects of salicylic acid on wheat salt sensitivity. *African Journal of Biotechnology*. 10(30): 5713–5718.
- Ergün, N., Öncel, I., 2012. Effects of some heavy metals and heavy metal hormone interactions on wheat (*Triticum aestivum* L. cv. Gun 91) seedlings. *African Journal of Agricultural Research*. 7(10): 1518–1523.
- Evans, G.C., Hughes, A.P., 1962. Plant growth and the aerial environment on the computation of unite leaf rate. *New Phytologist*. 61: 322–327.
- Gallego, S.M., Benavides, M.P., Tomaro, M.L., 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science*. 121(2): 151–159.
- Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*. 59: 309–314.
- Ghosh, S., Mitra, S., Paul, A. 2015. Physicochemical studies of sodium chloride on Mungbean (*Vigna radiata* L. Wilczek) and its possible recovery with spermine and gibberellic acid. *The Scientific World Journal*. 2015: 1–8. <http://dx.doi.org/10.1155/2015/858016>.
- Hayat Bhatti, K., Anwar, S., Nawaz, K., Hussain, K., Siddiqi, E.H., Sharif, R.U., Talat, A., Khalid, A., 2013. Effect of

- heavy metal lead (Pb) stress of different concentration on wheat (*Triticumaestivum* L.). *Middle-East Journal of Scientific Research*. 14(2): 148–154.
- Howladar, S.M., 2014. A novel *Moringaoleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. *Ecotoxicology and Environmental Safety*. 100: 69–75.
- Hussain, A., Abbas, N., Arshad, F., Akram, M., Khan, Z.I., Ahmad, K., Mansha, M., Mirzaei, F., 2013. Effects of diverse doses of lead (Pb) on different growth attributes of *Zea Mays* L. *Agricultural Sciences*. 4(5): 262–265.
- Khan, A.L., Hamayun, M., Kim, Y.H., Kang, S.M., Lee, J.H., Lee, I.J., 2011. Gibberellins producing endophytic *Aspergillusfumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. *Process Biochemistry*. 46: 440–447.
- Johansson L.H. and Borg L.A. H., 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. *Analytical Biochemistry*. 174(1): 331–336.
- Jomová, K., Morovič, M., 2009. Effect of Heavy Metal Treatment on Molecular Changes in Root Tips of *Lupinusluteus*L.. *Czech Journal Food Science*. 27: 386–389.
- Lichtenthaler, H.K., 1987. Chlorophyll and carotenoid: pigment of photosynthetic biomembranes. *Method in Enzymology*. 148: 350–382.
- Liu, Z.J., Zhang, X.L., Bai, J.G., Suo, B.X., Xu, P.L., Wang, L., 2009. Exogenous paraquat changes antioxidant enzyme activities and lipid peroxidation in drought-stressed cucumber leaves. *ScientiaHorticulturae*. 121: 138–143.
- Mathieu, A.S., Lutts, S., Vandoorne, B., Descamps, C., Périlleux, C., Dielen, V., Van Herck, J.C., Quinet, M., 2014. High temperatures limit plant growth but hasten flowering in root chicory (*Cichoriumintybus*) independently of vernalisation. *Journal of Plant Physiology*. 171(2): 109–118.
- Mittal, S., Kumari, N., Sharma, V., 2012. Differential response of salt stress on *Brassica juncea*: Photosynthetic performance, pigment, proline, D₁ and antioxidant enzymes. *Plant Physiology and Biochemistry*. 54: 17–26.
- Miteva, E., Hristova, D., Nenova, V., Maneva, S., 2005. Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, peroxidase activity and chloroplast pigments. *ScientiaHorticulturae*. 105(3): 343–358.
- Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M., 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*. 32(2): 429–448.
- Ozturk, F., Duman, F., Leblebici, Z., Temizgul, R., 2010. Arsenic accumulation and biological responses of watercress (*Nasturtium officinale* R. Br.) exposed to arsenite. *Environmental and Experimental Botany*. 69(2): 167–174.
- Piotrowska-Niczyporuk, A., Bajguz, A., Zambrzycka, E., Godlewska-zy1kiewicz, B., 2012. Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris*(*Chlorophyceae*). *Plant Physiology and Biochemistry*. 52: 52–65.
- Polizzotto, M.L., Lineberger, E.M., Matteson, A.R., Neumann, R.B., Badruzzaman, M., Ashraf-Ali, M., 2013. Arsenic transport in irrigation water across rice-field soils in Bangladesh. *Environmental Pollution*. 179: 210–217.
- Rastgoo, L., Alemzadeh A., 2011. Biochemical responses of Gouan (*Aeluropuslittoralis*) to heavy metals stress. *Australian Journal of Crop Science*. 5(4): 375- 383.
- Ratushnyak, A.Y., Ratushnyak, A.A., Andreeva, M.G., Kayumov, A.R., Trushin, M.V., 2012. Effect of lead and salicylic acid on some plant growth parameters in *Pisumsativum*L.. *European Journal Applied Sciences*. 4(2): 87–89.
- Rezaei, H., Ghorbanli, M., Peivandi, M., Pazoki, A.R., 2013. Effect of drought interactions with ascorbate on some biochemical parameters and antioxidant enzymes activities in *Dracocephalummoldavica* L., *Middle-East Journal of Scientific Research*. 13 (4):522- 531.

- Rintala, E.M., Ekholm, P., Koivisto, P., Peltonen, K., Venalainen, E.R., 2014. The intake of inorganic arsenic from long grain rice and rice-based baby food in Finland-Low safety margin warrants follow up. *Food Chemistry*. 150: 199–205.
- Sánchez-Rodríguez, E., Rubio-Wilhelmi-Mdel, M., Blasco, B., Leyva, R., Romero, L., Ruiz, J.M., 2012. Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought-sensitive cultivars in tomato under water stress. *Plant Science*. 188: 89–96.
- Shaddad, M.A.K., Hamdia-Abd-El-Samad, M., Mohammed, H.T., 2011. Interactive effects of drought stress and phytohormones or polyamines on growth and yield of two M (*Zea mays* L.) genotypes. *American Journal of Plant Sciences*. 2(6): 790–807.
- Siddiqui, M.H., Al-Wahaibi, M.A., Basalah, M.O., 2011. Interactive effect of calcium and gibberellin on nickel tolerance in relation to antioxidant systems in *Triticumaestivum*L.. *Protoplasma*. 248(3): 503–511.
- Singh, N., Ma, L.Q., Vu, J.C., Raj, A., 2009. Effects of arsenic on nitrate metabolism in arsenic hyperaccumulating and non-hyperaccumulating ferns. *Environmental Pollution*. 157: 2300–2305.
- Swarnakar, A. 2014. Induction of oxidative stress and osmolyte accumulation in response to sodium arsenate toxicity in Mungbean Seedlings and Its Amelioration. *Research Journal of Chemical and Environmental Sciences*. 2: 61-67.
- Szöllösi, R., 2014. Superoxide dismutase (SOD) and abiotic stress tolerance in plants: an overview. Oxidative damage to plants antioxidant networks and signaling. Chapter 3. India, pp. 89–129.
- Tuna, A. L., Kaya, C., Dikilitas, M., Higgs, D., 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany*. 62(1): 1–9.
- Vassilev, A., Lidon, F.C., Ramalho, J.C., Matos, Md.C.,Bareiro, M.G., 2004. Shoot cadmium accumulation and photosynthetic performance of barley plants exposed to high cadmium treatments. *Journal of Plant Nutrition*. 27(5): 775–795.
- Wang, P., Zhang, S., Wang, C., Lu, J., 2012. Effects of Pb on the oxidative stress and antioxidant response in a Pb bioaccumulator, *Vallisnerianatans*. *Ecotoxicology and Environmental Safety*. 78(1): 28–34.
- Yusuf, M., Hasan, S.A., Ali, B., Hayat, S.,Fariduddin, Q., Ahmad, A., 2008. Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. *Journal of Integrative Plant Biology*. 50(9): 1096–1102.
- Zhang, F.Q., Wang, Y.S., Lou, Z.P., Dong, J.D., 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandeliacandel* and *Bruguieragymnorrhiza*). *Chemosphere*. 67(1): 44–50.
- Zhu, X.F., Jiang, T., Wang, Z.W., Lei, J.G., Shi, Y.Z., Li, G.L., Zheng, S.J., 2012. Gibberellic acid alleviates cadmium toxicity by reducing nitric oxide accumulation and expression of IRT1 in *Arabidopsis thaliana*. *Journal of Hazardous Materials*. 239–240: 302–307.

تأثير استخدام زرنبيخ الصوديوم وحمض الجبريليك على النمو ونشاط الانزيمات المانعة للأكسدة وصبغات التمثيل الضوئي في صنفين من الأرز

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ملخص

تم اجراء هذه التجربة لدراسة تأثيراستخدام حمض الجبريليك وزرنبيخ الصوديوم على نشاط الانزيمات المانعة للأكسدة ومحتوى البروتين الذائب والنمو وصبغات التمثيل الضوئي (كلوروفيل "أ" وكلوروفيل "ب"، والكلوروفيل الكلي والكاروتينويدات) في صنفى الأرز "تاروم" و"شيوودي". تم معاملة البادرات بتراكيز مختلفة من زرنبيخ الصوديوم (صفر، 50 و 100 ملي مولر) وحمض الجبريليك (صفر و 100 ملي مولر) لمدة اسبوعين. بينت النتائج ان المعاملة بزرنبيخ الصوديوم زادت معنويا نشاط الانزيمات المانعة للأكسدة ومحتوى البروتين بينما خفضت النمو ومحتوى صبغات التمثيل الضوئي (كلوروفيل"أ" وكلوروفيل "ب"والكاروتينويدات) في كلا الصنفين. كما أظهرت النتائج ان حمض الجبريليك زاد معنويا نشاط الانزيمات المانعة للأكسدة ومحتوى البروتين والنمو وصبغات التمثيل الضوئي (كلوروفيل "أ" وكلوروفيل "ب"والكاروتينويدات) في النباتات المعاملة بزرنبيخ الصوديوم في صنفى الأرز. يظهر من النتائج أن المعاملة بحمض الجبريليك زادت مقاومة النباتات المعاملة بزرنبيخ الصوديوم في كلا الصنفين.

الكلمات الدالة: الأرز، زرنبيخ الصوديوم، حمض الجبريليك، نشاط الانزيمات المانعة للأكسدة، صبغات التمثيل الضوئي، البروتين الذائب.

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